Vol. LXVI, No. 2 April, 1934

THE

BIOLOGICAL BULLETIN

PUBLISHED BY THE MARINE BIOLOGICAL LABORATORY

BIOLOGICAL EFFECTS OF HEAVY WATER

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The observations of Lewis (1933) on the germination of seeds and of Taylor, Swingle, Eyring, and Frost (1933) on various fresh water animals have indicated that concentrated deuterium water or heavy water is toxic for, or at least unfavorably affects living matter. The cause of this action is at present unknown, but certain possibilities can be eliminated by the experiments which are reported in this paper. It is obvious that a mass of information must be gathered before interpretation of results becomes possible, and the following observations are frankly exploratory.

The water used was 95 to 97 per cent heavy water, kindly supplied by Professor H. S. Taylor of the Chemistry Department.

Because of the extreme sensitivity of living organisms to small amounts of impurities of various kinds, great attention was paid to the purification and testing of the samples of heavy water used. In the various stages of preparation of heavy water from electrolysis and burning of the hydrogen and oxygen formed, there is possibility of oxidized hydrocarbons, possibly aldehydes (from ozone acting on rubber tubing), being formed and also readily detectable amounts of nitrous acid from the oxyhydrogen flame burning in air. Water so formed is toxic to *Paramœcia,* killing them instantly with discharge of trichocysts, whether it contains 97 per cent heavy water or only 0.2 per cent heavy water, an effect undoubtedly due to its acidity. If redistilled from alkali alone or from alkali and $KMnO_4$, the 0.2 per cent heavy water is harmless while the 97 per cent heavy water kills *Paramœcium* in 6 to 15 hours, without discharge of trichocysts. The 0.2 per cent heavy water formed by oxyhydrogen combustion and redistilled from alkali and permanganate is also non-toxic to *Euglena, Amœba, Epistylis,* luminous bacteria and other small organisms, so that it appears reasonably certain that any effects of concentrated heavy water treated in the

same way must be due to the deuterium it contains and not to unknown impurities. There is always a possibility of doubt that minute traces of some unknown impurity may be present in a minimal effective concentration, but all known precautions have been taken to remove such. In the experiments reported below, heavy water redistilled from alkali has always been used and in most cases heavy water redistilled from alkali and permanganate.

Effect on Luminescence

The dry powdered luminous organism, *Cypridina*, when added to 97 per cent heavy water lumineses just as brightly and with the same color as in distilled water.

A suspension of luminous bacteria[1] (*Vibrio phosphorescens*, a fresh water form) lumineses about as brightly in approximately 85 per cent heavy water as in distilled water and the light lasts for over 24 hours in each, becoming less and less bright. After five days the bacteria in both heavy and distilled water, when inoculated in culture media in ordinary water, grew and luminesced normally. When inoculated in culture media in ordinary water after 41 days in heavy water, the bacteria also grew and luminesced normally. It is quite evident that the heavy water does not kill these forms.

Experiments with a salt water bacterium, isolated from squid at Woods Hole, Mass., added to heavy water plus salt (about 85 per cent final concentration), showed a marked diminution of luminescence in the 85 per cent heavy water after 4 hours as compared with ordinary water. In another experiment 63 per cent heavy water dimmed in 3 hours, but 36 per cent heavy water did not appreciably. The luminescence of the salt water bacterium is undoubtedly affected in the heavy water.

Effect on Growth of Luminous Bacteria

The next experiment was devised to find out if luminous bacteria would grow in a culture medium made up in 97 per cent heavy water. Two kinds of bacteria were also used, the fresh water form (*Vibrio phosphorescens*) and the salt water form. The sterile culture medium consisted of: 23 mg. bactonutrient agar, 10 mg. glycerine, 1 mg. $CaCO_3$ per cc. For the salt water form 30 mg. table salt was added, and for the fresh water form 3 mg. table salt. In one experiment with the fresh water form there was some growth and luminescence, while in two other experiments there was some growth but none or very faint luminescence.[2]

[1] I am deeply indebted to Mr. I. M. Korr for cultures of both forms of luminous bacteria.

[2] Frequently luminous bacteria will grow but will not luminesce under adverse conditions such as treatment with ultra-violet light or high temperatures (38°).

There was good growth and luminescence on the ordinary water controls.

Using the salt water form, in three experiments carried out at different times, a good diffuse [3] luminescent growth occurred in the 94–97 per cent heavy water medium, whereas a bright streak of growth and luminescence appeared on the ordinary water medium. After 24 days bacteria were transplanted from the heavy and ordinary water media to new ordinary water culture media and they grew and luminesced.

The conclusions are that luminous bacteria will grow slowly and one species will luminesce in 97 per cent heavy water, but not as well as in ordinary water, and that they are not killed by long contact with heavy water.

Effect on Protozoa and Small Organisms

Although *Paramœcium* and *Euglena* were studied by Taylor, Swingle, Eyring and Frost (1933), new samples of 95 per cent, 97 per cent and 100 per cent heavy water were tested on these forms and others to see if various samples of heavy water would give the same effects. It was found that they did. The experiments were carried out in hanging drops on cover glasses on depression slides sealed with vaseline. The drop of heavy water was mixed with a minute drop of water containing the organisms. This dilutes the 97 per cent heavy water to an unknown amount, possibly to 85–90 per cent.

Paramœcia almost immediately give the avoiding reaction and swim slowly in the heavy water, soon appearing somewhat bloated, and in two hours the contractile vacuoles are enormously enlarged and fail to empty. The well-known blisters appear at the surface. The animals are dead and disintegrated in less than 24 hours, while controls in distilled water are normal after 5 days.

One experiment was run with 100 per cent heavy water (somewhat diluted by the *Paramœcia*). They were killed in 6 to 10 hours with a similar sequence of events, while they live for days in 0.2 per cent heavy water. *Paramœcia* never recover in ordinary water when once disintegrated.

Amœba dubia and two rotifers, *Monostyla bulba*, and *Philodina roscola*, in heavy water are killed in from 6 to 20 hours. The *Amœbæ* round up, show no movement (and no large contractile vacuole) and look disintegrated in about six hours, whereas in distilled water, the *Amœbæ*, although rounded up at first, are quite normal after 48 hours, as are also the rotifers. The same statement applies also to another large species of *Amœba* and to a *Vorticella*-like infusorian, *Epistylis*, which almost immediately withdraws its peristome, the cilia beat more

[3] Although a streak inoculation was made.

and more slowly and soon all movement ceases and disintegration occurs. Controls are normal after 48 hours.

The conclusion is that a large number of small organisms are killed in 85–90 per cent heavy water and the more rapidly the greater its concentration.

Recovery of Euglenæ

Euglena gracilis in 97 per cent (slightly diluted) heavy water mostly rounds up and remains immobile for days. On the ninth day, the heavy water was replaced by distilled water and most of the *Euglenæ* recovered and swam about perfectly normally.

Euglena proxima[4] also shows the avoiding reaction, (euglenoid shapes), mostly round up and become immobile in heavy (90 per cent) water, but a certain percentage may be motile for three days although not nearly as active nor as elongate as the controls in ordinary water or water containing 0.2 per cent D_2O. After three days movement in practically all the organisms has ceased. After five days ordinary water was added to greatly dilute the heavy water and most of the *Euglenæ* recovered completely.

The conclusion is that *Euglenæ* are not irreversibly injured by the heavy water.

Effect on Protoplasmic Rotation

One-half of an *Elodea* leaf, showing marked protoplasmic rotation, was placed in 95 per cent heavy water at 25° C. A slowing of rotation occurs at first which very soon begins again, and in 15 minutes rotation is nearly as marked as in the half control leaf in ordinary distilled water at 25°. The heavy water was then replaced by more 95 per cent heavy water and this was again replaced after two hours so that the leaf must finally have been exposed to approximately 95 per cent heavy water with no dilution. The rotation gradually slows over a period of hours and there is still some slow rotation in some cells after 5 hours but much less than in the control. After 24 hours there was still some slow rotation in the leaf in heavy water but practically none in the control in distilled water, but rotation began again later. Another experiment gave a similar result. The cells look quite normal in appearance.

The conclusion is that a gradual slowing of protoplasmic rotation occurs in concentrated heavy water but that it is not marked or rapid and that *Elodea* cells may show rotation and are not injured by heavy water after 24 hours.

[4] Kindly supplied in pure culture by Dr. R. Glaser of the Rockefeller Institute, Princeton, New Jersey.

Penetration of Heavy Water into Cells

In order to test penetration in a rough way, *Elodea* leaf cells were plasmolyzed in m/2 cane sugar solution and then returned to heavy water, when the protoplasts reëxpand again. The heavy water must have entered the *Elodea* cells to again restore osmotic equilibrium. This method is not sufficient to detect differences in rate of entrance as compared with ordinary water, which should be carried out on the swelling of spherical cells such as *Arbacia* eggs in a hypotonic medium. Effects of heavy water cannot be attributed to non-penetration. Indeed the enormous enlargement of the contractile vacuoles in *Paramœcium* in heavy water points to an interference with the water-eliminating mechanism rather than an influence on water penetration. However, such an interference may be brought about by many unfavorable conditions.

Does H_2O_2 Accumulate in Heavy Water?

In view of observations of Taylor and Pace[5] that heavy water retards the action of liver catalase on H_2O_2, experiments were carried out to determine if the effect of heavy water is so to retard the action of catalase in cells as to cause possible accumulation of H_2O_2 as a result of respiration or possibly of photosynthesis. In either case the destructive effect might be due to accumulation of H_2O_2.

This theory can be tested in three ways. First, by finding if heavy water is toxic for anærobic forms that contain no catalase. Second, by testing ærobic forms, which can also live under anerobic conditions for some time, in absence of oxygen. Third, by testing green organisms like *Euglena* in light and in darkness.

Euglena proxima or *E. gracilis* was placed in 85–90 per cent heavy water on slides in hanging drops and kept both in darkness and in light. Since this concentration of heavy water does not immediately affect all the organisms but merely causes many of them to become spherical and immobile, while others move about slowly, any additional H_2O_2, possibly accumulating in light from photosynthetic processes, might be sufficient to cause a difference in behavior of the organism in light and in darkness. However, no significant difference could be found, although many experiments were performed. In heavy water spherical and immobile *Euglenæ* do not become active in the dark. After three days all the *Euglenæ* were immobile and spherical whether they had been kept in light or in darkness. In general, *Euglenæ* are more active in the dark, distributing themselves uniformly through the drop, whereas in the light they collect in one spot, but this is a light reaction. It occurs in ordinary

[5] Private communication from Professor H. S. Taylor.

water also and cannot be connected with the possible accumulation of H_2O_2.

Paramœcium cannot be tested in heavy water in a pure hydrogen atmosphere because *Paramœcium* in culture medium will not withstand absence of oxygen for any length of time. They are mostly killed in one hour in a hanging culture drop in a pure hydrogen atmosphere.

However, if *Paramœcia* are killed by the accumulation of H_2O_2 in heavy water, the effects of H_2O_2 on *Paramœcia* should be the same as the effects of heavy water. To test this, Merck's superoxol (a very pure preparation of H_2O_2) was added to culture medium containing *Paramœcium* in a concentration which caused disruption and surface blister formation in 1 to 1.5 hours. Only a slight enlargement of the contractile vacuole occurred, but not the enormous enlargement characteristic of a heavy water effect. This may be taken as additional evidence against the view that accumulating H_2O_2 is responsible for the toxic effects on *Paramœcium*.

Summary

Heavy water (85–95 per cent) has been found not to prevent the luminescence of dried *Cypridina* nor to affect the luminescence of a fresh water luminous bacterium but to diminish the luminescence of a marine form; to retard growth of luminous bacteria, sometimes allowing slow growth without luminescence; to kill a number of protozoa and rotifers, but not to kill bacteria and not to injure *Euglena* irreversibly; to affect *Euglena* equally in light and in the dark; to affect only slowly protoplasmic rotation of *Elodea* cells and to penetrate into *Elodea* cells. In view of the slow and often reversible effects of heavy water, its action may be likened to that of a generally unfavorable environment, leading to progressive changes in the cell. No more can be said at present than to suggest that these changes are the result of differential effects on the rate of biochemical reactions which ordinarily proceed at a certain definite rate in relation to each other.

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